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REGRESSIONS FOR ESTIMATING GASTROPOD BIOMASS WITH MULTIPLE SHELL METRICS

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INTRODUCTION

Snails are among the most important primary consumers in freshwater ecosystems. They eat large amounts of periphyton, facilitate nutrient regeneration for the resources they consume, and in ecosystems with greater than two trophic levels, snails are a vital conduit for energy flow to upper trophic levels by serving as prey to numerous invertebrate and vertebrate predators (Dillon, 2000). Estimates of individual biomass (mass/individual) enable calculations of standing stock (mass/area) and production (mass/area/time). These measures are superior to density (no./area) for assessing the influence of animals on communities and ecosystems because they incorporate size-structured aspects of energy flow (Regester et al., 2006; Rosas-Luis et al., 2008).

Length-mass regressions enable quick estimation of individual biomass to assist estimating standing stock and production. Regressions provide mass estimates without sacrificing animals, which may not be feasible for protected taxa or desirable because repeatedly sampling the same population can cause bias (Cahill et al., 2002). Obtaining biomass data from preserved specimens is facilitated by regressions because length is a relatively stable measure compared to mass (Wetzel et al., 2005). However, historically most studies of freshwater snails report density without measures of biomass (Garner & Haggerty, 2010; Ruehl & Trexler, 2011). Among those studies that report biomass data, few report regressions that include more than one measure of length (e.g., shell length or width) or mass (e.g., wet mass or ash-free dry mass, a measure of organic content) and rarely combine multiple measures of length to improve mass estimates (e.g., Eckblad, 1971; Boerger, 1975; Collins, 1992; Benke et al., 1999; González et al., 2002; Deichmann et al., 2008). Combining multiple length measures should improve predictive power and provide more accurate biomass estimates for snails because they exhibit a diversity of shapes. Comparisons among possible regression models may reveal that different metrics or combinations of metrics are better predictors of a particular mass measure for a given group (e.g., AFDM vs. wet mass).

In this study, we consider three common, structurally diverse freshwater snail genera *Planorbella*, *Pomacea* and *Haitia* to examine the utility of using multiple length measures to predict multiple soft-tissue mass measures. *Planorbella* (Planorbidae) are pulmonate snails with planispiral shells that grow sinistrally (left handed). *Haitia* (Physidae) are small pulmonates with a sinistrally growing conispiral shell. *Pomacea* (Ampulariidae) are large caenogastropods that have globose, conispiral shells that are generally dextral (right handed) with an operculum. Additionally, we report the first length-to-mass regression for *Pomacea flagellata* Say, 1827.

METHODS

We collected *P. duryi* Wetherby, 1879 (7.67–23.46 mm shell length, *n* = 89) and *H. cubensis* Pfeiffer, 1839 (4.58–9.25 mm shell length, *n* = 30) from populations maintained at the Daniel Beard Research Center in Everglades National Park. These populations originated from collections made throughout the Everglades and were supplemented annually with wild-caught individuals. *Pomacea paludosa* Say, 1829 (10.33–44.43 mm, *n* = 19) were obtained from live-caught animals and their offspring. *Pomacea paludosa* sample size was comparatively small, but we believe it is sufficient for our purpose because preliminary regressions indicated that model fit was very good. *Pomacea flagellata* (6.8–51 mm, *n* = 49) were collected from Mexico (Sian Kaan, Quitana Roo, 19.20°N, 87.89°W) and Belize

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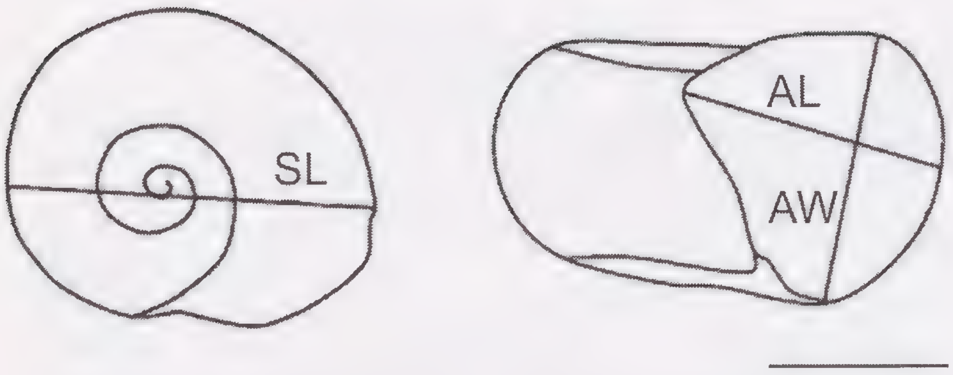

FIG. 1. Graphical description of how aperture width and length, and shell length were measured *P. duryi*. Shell length is the lateral distance from anterior to posterior, aperture length is the distance across the aperture from anterior to posterior, aperture width is the length across the aperture at its widest point using calipers to 0.01 mm. Scale bar = 6 mm.

(Crab Catcher lagoon, Orange Walk 17.65°N, 88.62°W). Facilities were not available to get dry and ash mass of *P. flagellata*. All snails were sacrificed by freezing for 24 h (Nielsen & Gosselin, 2011).

Shell measurements were made to the nearest 0.01 mm with calipers and weighed to the nearest 0.0001 g. Shell length and aperture width and length were measured on *Planorbella* (Fig. 1) and *Haitia* (Fig. 2). We measured shell length, aperture width and length as well as operculum width and length on *P. paludosa* (Fig. 3). After measurements, the soft tissue was separated from the shell with forceps. Soft tissue and shell were weighed and then dried at 60°C to a constant mass. Dried soft tissue, shell, and opercula were weighed and then combusted at 500°C for four hours in a muffle furnace and reweighed. We subtracted the ashed mass from the dried mass to calculate AFDM.

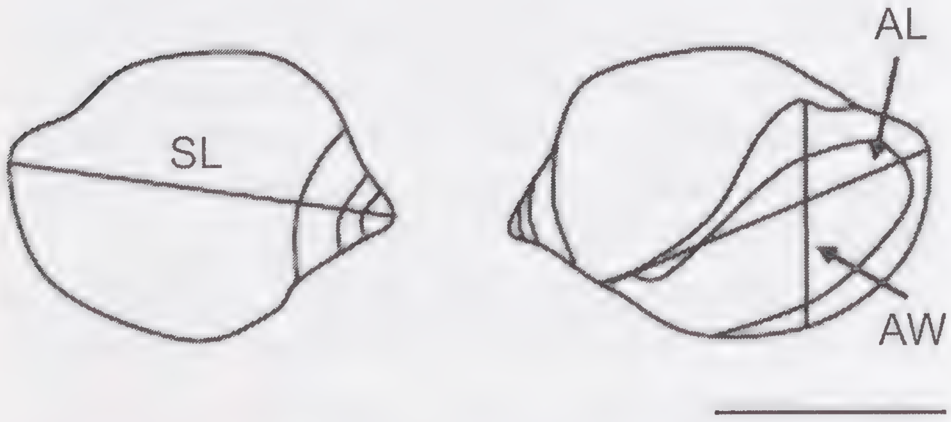

FIG. 2. Graphical description of how aperture width and length, and shell length were measured for *H. cubensis*. Shell length is the lateral distance from anterior to posterior, aperture length is the distance across the aperture from anterior to posterior, and aperture width is the length across the aperture at its widest point using calipers to 0.01 mm. Scale bar = 3 mm.

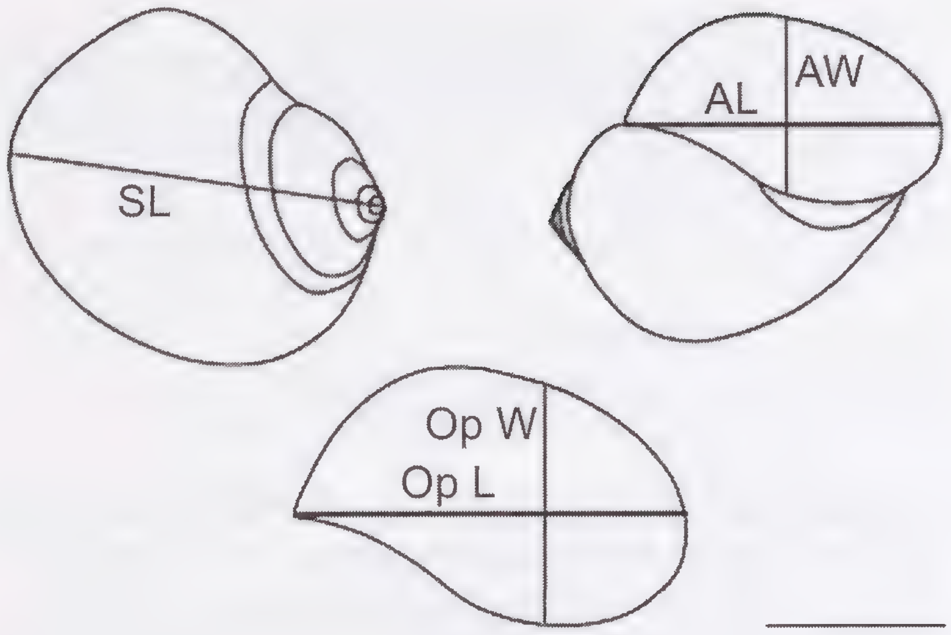

FIG. 3. Graphical description of how aperture and operculum width, length, and shell length were measured for *P. paludosa* and *P. flagellata*. Shell length is the lateral distance from anterior to posterior, aperture length is the distance across the aperture from anterior to posterior, aperture width is the length across the aperture at its widest point and opercula length and width that correspond with aperture length and width measurements using calipers to 0.01 mm. Scale bar = 10 mm.

We quantified the capacity for multiple length measures to predict mass compared to single measures using a model selection procedure with regression. Regressions were used to determine relationships between mass (wet, dry, and AFDM) and shell size (length, width, operculum length, operculum width). We tested all metric combinations to find the best model for each measure of mass for each species. Models were compared using the information-theoretic approach proposed by Burnham & Anderson (2002). Each model was ranked relative to the others using second order Akaike's Information Criterion for small sample sizes (AIC_c) where the lowest value indicated the best fit. The AIC_c calculation adds another parameter to the AIC formula that further penalizes model fit for including more independent variables. Models were considered equal if the difference between the calculated AICc value and the lowest AICc value for each dataset was ≤ 2 (Compton et al., 2002). Model weights facilitated better resolution of models with AICc values ≤ 2. Data were natural-log (ln) transformed because a plot of mass by length suggested a power relationship.

RESULTS AND DISCUSSION

The information-theoretic approach revealed that in many cases, multiple measures of shell morphology predicted tissue mass better than single shell measurements. Model weights, the relative probability of model accuracy, indicated that inclusion of a length and width parameter provided more robust models (Table 1). Even when the ΔAICc values for models with only one parameter were low, the model weight was correspondingly low meaning that it was unlikely that single parameter models provided the best fit. Inclusion of extra parameters also increased the R^2 value (Table 2). For the two pulmonate species, the best combination of measurements changed depending on the mass measurement, but always included shell length. Wet mass was predicted best by shell length and aperture length or width for *P. duryi* (Table 1). Similarly, a combination of shell length and aperture width, or these and aperture length, predicted wet mass best in *H. cubensis*. However, models that included only shell length and aperture width predicted dry mass best for *P. duryi*, while shell length with aperture length best predicted dry mass in *H. cubensis*. Finally, a combination of shell length and aperture width best predicted AFDM for both *P. duryi* and *H cubensis*. Therefore, regardless of mass measurement, we recommend gathering data on shell length and ap-

TABLE 1. Lowest AIC values out of all possible model variations including shell length (SL), aperture width (AW), aperture length (AL), operculum length (OpL) and operculum width (OpW) for each species and mass measure. Multiple models are included if ΔAIC ≤ 2.5. Model weight is the relative likelihood a given model provides the best fit.

Species	Mass Measure	Parameters	ΔAICc	Model Weight
P. duryi	Wet	SL, AW, AL	0	0.65
	Wet	SL, AL	2.12	0.22
	Dry	SL, AW	0	0.61
	Dry	SL, AW, AL	2.21	0.2
	AFDM	SL, AW	0	0.63
	AFDM	SL, AW, AL	2.28	0.2
H. cubensis	Wet	SL, AW	0	0.71
	Wet	SL, AW, AL	1.91	0.27
	Dry	SL, AL	0	0.68
	Dry	SL, AL, AW	2.4	0.2
	AFDM	SL, AW	0	0.3
	AFDM	SL	0.16	0.28
	AFDM	SL, AL	0.35	0.25
	AFDM	SL, AW, AL	1.62	0.13
P. paludosa	Wet	OpL	0	0.36
	Wet	AL, OpL	2.4	0.11
	Dry	AW, OpL	0	0.26
	Dry	OpL	1.31	0.13
	Dry	AW, AL	1.39	0.13
	AFDM	AW, OpL	0	0.35
	AFDM	OpL	2.62	0.09

TABLE 2. Top two regression equations as selected by AIC and the equation for shell length (SL) to provide a comparison with the exception of *H. cubensis* AFDM where SL was one of the top two regression equations. Regressions are of transformed SL, aperture width (AW), and length (AL) and operculum width (OpW) and length (OpL) to ln transformed wet mass, dry mass and AFDM for *P. duryi* (n = 89), *P. paludosa* (n = 19) and *H. cubensis* (n = 30).

Mass	*P. duryi*	*P. paludosa*	*H. cubensis*
Wet	1.16 SL + 0.66 AW + 0.77 AL - 7.07	3.09 OpL - 7.97	2.14 SL + 0.87 AW - 8.96
	$R^2 = 0.87$	$R^2 = 0.98$	$R^2 = 0.73$
	1.31 SL + 1.1 AL - 6.94	0.88 AL + 2.23 OpL - 7.99	2.37 SL + 0.96 AW - 0.36 AL - 8.96
	$R^2 = 0.86$	$R^2 = 0.98$	$R^2 = 0.73$
	2.45 SL - 7.96	2.85 SL - 8.33	2.83 SL - 9.34
	$R^2 = 0.84$	$R^2 = 0.97$	$R^2 = 0.67$
Dry	1.72 SL + 0.8 AW - 9.49	-1.62 AW + 4.51 OpL - 9.43	1.91 SL + 0.36 AL - 9.31
	$R^2 = 0.81$	$R^2 = 0.92$	$R^2 = 0.68$
	1.65 SL + 0.75 AW + 0.1 AL - 9.41	2.89 OpL - 9.01	1.89 SL + 0.1 AW - 1.44 AL - 12.06
	$R^2 = 0.80$	$R^2 = 0.91$	$R^2 = 0.67$
	2.28 SL - 9.66	2.65 SL - 9.34	3.13 SL - 12.18
	$R^2 = 0.80$	$R^2 = 0.89$	$R^2 = 0.64$
AFDM	1.53 SL + 1.34 AW - 10.27	-1.33 AW + 4.32 OpL - 9.92	2.01 SL + 0.54 AW - 10.81
	$R^2 = 0.66$	$R^2 = 0.96$	$R^2 = 0.5$
	1.54 SL + 1.35 AW - 0.02 AL - 10.29	3.0 OpL - 9.62	2.44 SL - 11.05
	$R^2 = 0.66$	$R^2 = 0.95$	$R^2 = 0.49$
	2.48 SL - 10.57	2.80 SL -10.08	
	$R^2 = 0.64$	$R^2 = 0.93$	

erture width for species that are shaped like *P. duryi* or *H. cubensis*, and adding the aperture length measure to increase accuracy. Adding this extra measure requires little effort but provides three-dimensional information on shell shape and improves biomass estimates.

Presence of predators and variable nutrient concentrations often alter shape and life history traits that may influence length-mass regressions. Predators induce different shell shapes, shell thickness, and life history traits in both physid and planorbid snails (DeWitt et al., 2000; Hoverman et al., 2005; Auld & Relyea, 2008; Ruehl & Trexler, 2013). Differential allocation of resources represents a tradeoff with reproductive effort and is situation dependent with a size limited predator: in times of low resources, individuals will mature early and risk predation to reproduce quickly, while in times of abundant resources individuals will attempt to grow to a size refuge before reproducing (Chase, 1999; Crowl & Covich, 1990). Snails that differentially allocate resources or induce shape variation may affect the fit of length-weight regressions by adding variability. For example, tissue mass within a globose shell may weigh the same as tissue from a longer shell but not be consistent with the relationship between the two established by the regression. A similar case might exist if more resources were allocated to shell thickness than tissue growth. Caution should be exercised when applying these regressions to samples taken in areas with only one predator type or during extremes in resource availability (e.g., eutrophication, drought). Despite this limitation, these general regressions are applicable for studies calculating energy budgets for ecosystems or energy flow in food webs.

Comparing regressions for *P. paludosa* revealed that operculum length alone or combined with aperture width or length yielded the lowest AICc for all mass measures. While all three models were equivalent according to the AICc value (Table 1), model weights indicated OpL had the best fit for wet mass, OpL and AW gave the best fit for dry mass and AFDM. Therefore, for two of the metrics multiple measures were the best predictors.

Some *Pomacea* species have shown sexual dimorphism (Cazzaniga, 1990) that may have affected the accuracy of our regressions. However, the reported R^2 values were higher than those found in the literature: Sykes (1987) and Cattau et al. (2010) documented a R^2 of 0.75 and Bourne & Berlin (1982) found a maximum R^2 of 0.56. The regressions from this study may bias results if estimating mass for one gender, but this scenario is not likely for field collections because even though brood sex ratios are highly variable in *Pomacea* the population sex ratio is not (Yusa & Suzuki, 2002).

Youens & Burks (2008) found similar results to our *P. paludosa* regressions when comparing wet mass to shell height and shell length (they referred to our operculum length as operculum width). They agreed with Guedes et al. (1981) that operculum length is analogous to the base of an elaborate shell and therefore represents the most reliable measure of wet mass. Another possible explanation is that because the operculum grows from the tissue, it is more likely to be indicative of body mass. Use of operculum length for mass estimation has ancillary benefits. For example, intact opercula are easily retrieved from gut contents of molluscivores; a regression that predicts mass from operculum length provides an estimate of energy transfer through the food web and offers potential insight into size-selective predation by different molluscivores (Hulsey et al., 2006). Additionally, opercula are occasionally found in the fossil record and could provide estimates of standing stock for snails in an ecosystem from the past (Martín & Francesco, 2006). Aperture and operculum size are undoubtedly related and possibly redundant as factors, however our results show the value of including these parameters in our analysis. Models of best fit for all three mass measures include both operculum and aperture measurements. In addition, aperture and operculum length are the only parameters in one of the wet mass models. Because the AIC method penalizes additional parameters, these models would not have had low AIC scores unless both were necessary. Therefore, we felt the inclusion of both aperture and operculum measurements was important.

Only shell length and wet mass measures were available for *P. flagellata* ($y = 2.593x - 7.923$, $R^2 = 0.83$). We include this regression because it is the first reported length-mass relationship for this species. Though we do not have the same depth of information for this species as the others, the information we have reported will aid future studies on this species and the ecosystems where it lives.

The error structure in a non-linear model is assumed to be normal and additive while error structure in a log-transformed linear model is assumed to be lognormally distributed and multiplicative (Xiao et al., 2011). While there is some debate as to which error structure is most realistic for biological studies, use of log-transformed, multiplicative error structures have been found to be more reliable (Kerkhoff & Enquist, 2009; Xiao et al., 2011). Therefore we are confident in our use of log-transformed data.

Few gastropod studies report length-mass regressions for wet mass, dry mass and AFDM together (but see Collins, 1992), but studies that use biomass report data using each of the three measures. We report all three mass measures to facilitate application of the technique and because each measure potentially conveys different information. Wet mass provides an estimate of the live mass of the animal and is probably most useful when comparing among closely related taxa that have similar shapes. Dry mass includes both organic and inorganic matter in estimates and could be reported instead of wet mass when comparing among taxa that differ greatly in the water content of their tissue. Ash-free dry mass reveals the organic content of tissue, which is an indicator of the differential allocation of resources between hard and soft tissues within an individual or among taxa. Therefore, depending on the scope and level of taxonomic comparison, researchers might report different forms of mass measurements, but reporting all three regressions will enable the conversion of one to the other for meta-analytic purposes. Because snails exhibit a wide diversity of form, one single measurement is unlikely to be adequate for all taxa. To address this challenge, we recommend reporting multiple length measures that will improve comparisons among studies by increasing consistency and accuracy. Furthermore, we suggest research to examine the accuracy of these regressions to different species within genera to aid in determining the extent of their applicability. Finally, using multiple length measures simultaneously to predict biomass offers more accurate and precise estimates of snail mass to better predict the influence of snails on communities and ecosystems. We propose this study as a first step towards encouraging the use of multiple measures of shell length for predicting biomass that will facilitate estimates of standing stock and production in ecosystems for greater understanding of energy flow and ecosystem function.

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